AMENDMENTS TO THE CLAIMS

Applicants have amended claim 1 without any intention of disclaiming equivalents thereof, and have introduced new claim 48. The following list of claims replaces all prior versions and lists of claims in the application.

Listing of Claims:

1. (Currently Amended) A method for achieving high sensitivity detection and/or high accuracy quantitation of a plurality of target proteins in a biological sample, at least one of which comprises an expression product of an alternative splicing form of a single DNA, the method comprising the steps of:

(1) fragmenting proteins in the sample using a predetermined denaturation and proteolytic protocol to generate a solution of polypeptide analytes comprising sequences comprising peptide epitope tags (PETs) unambiguously indicative of the presence in the sample of the target proteins from which they are derived, at least one ~~of which is~~ PET being unambiguously indicative of the presence in the sample of the alternative splicing form protein from which it is derived and ~~comprises~~ comprising an amino acid sequence encoded by an RNA spanning a splice junction;

~~(2) generating a plurality of capture agents specific for respective said PETs and immobilizing said capture agents to a solid support to provide an addressable array of plural capture agents;~~

~~(3)~~ (2) contacting ~~said addressable array with~~ said solution with a plurality of capture agents specific for respective said PETs and immobilized on a solid support in an addressable array, at least one of said capture agents being specific for the at least one PET comprising an amino acid sequence encoded by an RNA spanning a splice junction, to yield captured polypeptide analytes bound by interaction between respective capture agents and said PETs presented by respective said polypeptide analytes; and,

~~(4)~~ (3) detecting the presence and amounts or absence of said target proteins in the sample, including at least said protein expression product of an alternative splicing form, by

detecting the presence and amounts or absence of said captured polypeptide analytes, including said at least one polypeptide analyte comprising an amino acid sequence encoded by an RNA spanning a splice junction, using secondary capture agents specific for sites separate from said PETs on the captured polypeptide analytes and labeled with a detectable moiety.

2. (Previously Presented) The method of claim 1 comprising providing an addressable array of capture agents comprising plural capture agents which selectively interact with different PETs from one of said target proteins and quantitating, if present, the amount of the target protein in the sample by averaging results obtained from each said capture agent.

3. (Previously Presented) The method of claim 1 wherein said capture agents comprise antibodies.

4. (Previously Presented) The method of claim 1, wherein said capture agents comprise a member selected from the group consisting of non-antibody polypeptide, PNA (peptide nucleic acids), scaffolded peptide, peptidomimetic compound, polynucleotide, carbohydrates, artificial polymers, plastibody, chimeric binding agent derived from low-affinity ligand, and small organic molecules.

5. (Canceled)

6. (Previously Presented) The method of claim 1, wherein a subset of said capture agents bind to one of said PETs.

7. (Previously Presented) The method of claim 1, wherein one of said target proteins has two or more different forms within said biological sample.

8. (Original) The method of claim 7, wherein said different forms include unprocessed / pro-form and processed / mature form.

9-12. (Canceled)

13. (Previously Presented) The method of claim 7, further comprising determining a percentage of one form of the one of said target proteins as compared to a total target protein, or

ratio of a first form of the one of said target proteins to a second form of the one of said target proteins.

14-18. (Canceled)

19. (Original) The method of claim 1, wherein said sample is a body fluid selected from: saliva, mucous, sweat, whole blood, serum, urine, amniotic fluid, genital fluid, fecal material, marrow, plasma, spinal fluid, pericardial fluid, gastric fluid, abdominal fluid, peritoneal fluid, pleural fluid, synovial fluid, cyst fluid, cerebrospinal fluid, lung lavage fluid, lymphatic fluid, tears, prostatitc fluid, extraction from other body parts, or secretion from other glands; or from supernatant, whole cell lysate, or cell fraction obtained by lysis and fractionation of cellular material, extract or fraction of cells obtained directly from a biological entity or cells grown in an artificial environment.

20. (Previously Presented) The method of claim 1, wherein said sample is obtained from human, mouse, rat, frog, fish, fly, nematode, fission or budding yeast, or plant.

21. (Original) The method of claim 1, wherein said sample is produced by treatment of membrane bound proteins.

22. (Canceled)

23. (Previously Presented) The method of claim 1, wherein each of said secondary capture agents is labeled with a detectable moiety selected from: an enzyme, a fluorescent label, a stainable dye, a chemilumninescent compound, a colloidal particle, a radioactive isotope, a near-infrared dye, a DNA dendrimer, a water-soluble quantum dot, a latex bead, a selenium particle, or a europium nanoparticle.

24. (Previously Presented) The method of claim 23, wherein at least one of said secondary capture agents is labeled with a fluorophore.

25. (Canceled)

26. (Previously Presented) The method of claim 1, wherein said sample contains billion molar excess of unrelated proteins or fragments thereof relative to one of said target proteins.

27. (Previously Presented) The method of claim 1, wherein said PETs are identified based on one or more protein sources selected from: sequenced genome or virtually translated proteome, virtually translated transcriptome, or mass spectrometry database of tryptic fragments.

28. (Previously Presented) The method of claim 1, wherein one or a combination of said target proteins serve as a biomarker.

29. (Canceled)

30. (Previously Presented) An array of capture agents for detecting and quantitating plural target splice variant proteins comprising the expression products of one or more splice variants of a single DNA within a biological sample, the array comprising a plurality of capture agents, each immobilized on a distinct addressable location on a solid support, plural of said capture agents specifically binding to recognition sequences comprising peptide epitope tags (PETs) displaying solvent accessible binding surfaces unambiguously indicative of the presence in the sample of the proteins from which they are derived, at least one of said PETs comprising an amino acid sequence encoded by an RNA spanning a splice junction.

31. (Previously Presented) The array of claim 30, wherein said solid support comprises beads or an array device comprising features disposed in a manner that encodes the identity of said capture agents disposed thereon.

32. (Previously Presented) The array of claim 30, comprising 2 - 100 or more different capture agents.

33. (Previously Presented) The array of claim 30, wherein one of said capture agents is a single chain antibody.

34. (Previously Presented) The array of claim 30, wherein one of said capture agents is an antibody or antigen binding portion thereof.

35-41. (Canceled)

42. (Previously Presented) A method for detecting in a biological sample the presence or absence of plural target proteins, at least some of which comprise an expression product of one or more splice variants of a single DNA, the method comprising the steps of:

(1) fragmenting proteins in the sample, using a predetermined denaturation and proteolytic protocol, to generate a solution of polypeptide analytes comprising recognition sequences unambiguously indicative of the presence in the sample of proteins from which they are derived, at least one of said recognition sequences comprising an amino acid sequence encoded by an RNA spanning a splice junction;

(2) contacting said solution with an addressable array comprising a plurality of capture agents, each of which specifically binds a recognition sequence, and at least one of which specifically binds the at least one recognition sequence comprising an amino acid sequence encoded by an RNA spanning a splice junction, to yield captured polypeptide analytes bound by interaction between respective capture agents and said recognition sequences presented by respective said polypeptide analytes; and,

(3) detecting the presence or absence of said target proteins in the sample by detecting the presence or absence of said captured polypeptide analytes, including said polypeptide analyte comprising a recognition sequence comprising an amino acid sequence encoded by an RNA spanning a splice junction, using secondary capture agents specific for available sites on respective said captured polypeptide analytes and labeled with a detectable moiety.

43. (Canceled)

44. (Previously Presented) The method of claim 42, wherein the addressable array of capture agents comprises plural capture agents which selectively interact with different recognition sequences from the same target protein in the sample.

45. (Previously Presented) The method of claim 44, further comprising quantitating, if present, the amount of the target protein in the sample by averaging results obtained from each said capture agent.

46. (Previously Presented) The method of claim 42, wherein said capture agents comprise antibodies.

47. (Previously Presented) The method of claim 6, wherein said subset of said capture agents have different affinity and/or avidity for the one of said PETs.

48. (New) A method for achieving high sensitivity detection and/or high accuracy quantitation of a plurality of target proteins in a biological sample, at least one of which comprises an expression product of an alternative splicing form of a single DNA, the method comprising the steps of:

(1) fragmenting proteins in the sample using a predetermined denaturation and proteolytic protocol to generate a solution of polypeptide analytes comprising sequences comprising peptide epitope tags (PETs) unambiguously indicative of the presence in the sample of the target proteins from which they are derived, at least one PET being unambiguously indicative of the presence in the sample of the alternative splicing form protein from which it is derived and comprising an amino acid sequence encoded by an RNA spanning a splice junction;

(2) contacting the solution with pairs of first and second capture agents respectively specific for the PETs and for sites separate from the PETs on the polypeptide analytes, one capture agent of each pair being immobilized on a solid support and the other being labeled with a detectable moiety, at least one of the first capture agents being specific for the at least one PET comprising an amino acid sequence encoded by an RNA spanning a splice junction, to yield captured polypeptide analytes bound by respective pairs of capture agents; and,

(3) detecting the presence and amounts or absence of said target proteins in the sample, including at least said protein expression product of an alternative splicing form, by detecting the presence and amounts or absence of labeled capture agents bound to respective polypeptide analytes, including said at least one polypeptide analyte comprising an amino acid sequence encoded by an RNA spanning a splice junction, bound to respective immobilized capture agents.